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Evaluation of a poly-herbal preparation for the treatment of peptic ulcer

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Abstract

The aim of this study was to validate the traditional uses of ulcerene, a poly-herbal formulation in ethanol, aspirin and stress-induced gastric ulcer model of rat. The extent of gastric ulcer formation was studied, using ulcer score, ulcer index, percentage cure through gross examination and histopathological evaluation. A significant ($p<0.001$) dose-dependent anti-ulcerant effect was observed in ulcerene (50 and 100 mg/kg)-treated group with highest effectiveness against ethanol-induced ulcer. The concentration-dependent spasmolytic effect was seen in spontaneously contracting, high K^+ (80 mM) and carbachol (1 µM)-induced jejunum contractions (10, 0.3 and 1 mg/mL), similar to dicyclomine (10, 1 and 3 µM), indicated non-specific spasmolytic mechanism behind the effect. By considering these results, ulcerene can be suggested for the treatment of peptic ulcer.

Introduction

Peptic ulcer is the lesion in the duodenal and gastric epithelium, associated with acute or chronic inflammation. Presently various synthetic anti-ulcerant drugs are available (Chioma et al., 2011). However, each of these drugs may cause severe adverse effects such as diarrhea (proton pump inhibitors), antiandrogenic effect (H_2 receptor blockers), constipation (sucralfate) and abortion in women (misoprostol) (Alam, 2013). High treatment cost and undesirable effects of the synthetic antiulcer drugs decrease the compliance and leads to the treatment failures. Therefore, there is need to search safe, effective and affordable alternatives options for the treatment of peptic ulcer (Ukwuani et al., 2012).

The vast majority of people still rely on the traditional medicines for their everyday healthcare needs (Griggs et al., 2001). These medicines are relatively safer and cheaper than synthetic or modern medicine (Mann et al., 2008).

The aim of this study was to evaluate the claimed traditional uses of ulcerene, a poly-herbal formulation and to find out its possible mechanism of action.

Materials and Methods

Plant material

Six gram of ulcerene contained *Bambusa arundinacea* (100 mg), *Coriandrum sativum (*100 mg), *Elettria cardamomum* (100 mg), *Foeniculum vulgare* (200 mg), *Rosa damascene* (200 mg), *Mineral bezoar triturated (*100 mg) and *Pistacia lentiscus* (50 mg).

Crude extract preparation

The product, ulcerene, was in the form of powder was collected from a local herbalist. The powder was macerated in 70% aqueous methanolic solution for three days at room temperature. It was shaken intermittently (Chaudhary et al., 2012). Macerate was filtered through double-layered muslin cloth followed by filtration through filtration paper. This procedure was carried out thrice to get maximum yield. The filtrate was then vaporized in a rotatory evaporator at low temperature (40-45°C) and pressure (-760 mmHg) to convert into a viscous mass. The semi-solid extract was stored in the refrigerator until used.

Drugs and chemicals

Chemicals and drugs of research and commercial grade

were obtained respectively. The drugs used were sucralfate (Pacific Pharmaceuticals Pvt Ltd.), ranitidine (GSK), misoprostol (Atco Laboratories Pvt Ltd.) and aspirin (Reckitt Benckiser). The chemicals used were acetylcholine, carbachol (Sigma-Aldrich Co., USA), potassium chloride, sodium chloride, calcium chloride, sodium bicarbonate, magnesium chloride, sodium dihydrogen phosphate, glucose and methanol (Merck, Germany). Stock solutions of standard drugs were prepared in distilled water and stored in a refrigerator. Physiological solution (Tyrode's solution), dilutions of standard drugs and extract were freshly prepared in distilled water on the day of the experiment.

Animals

Locally bred male rabbits (1.0-1.5 kg) and albino rats (200-250 g) of either sex were housed in the animal house of Faculty of Pharmacy, The University of Lahore. Animals were kept under controlled environment (23-25°C), were provided with standard diet and filtered water. Rabbits were sacrificed by the blow behind the neck after overnight fasting, but were given water *ad libitum*, before the experimental work. Rats were sacrificed using chloroform anesthesia.

In vivo experiments

Ulcer induction

For the induction of ulcer, ethanol- (1 mL/200 g) (Hollander et al., 1985), aspirin- (200 mg/kg) (Goel et al., 1986) and stress- (cold water swimming) (Brodie et al., 1962) induced methods were used with little modification. In each protocol animals (200-250 g) were divided into five groups, with five rats in each group. After overnight fasting, animals of all groups were administered ulcerogenic substance, except control group that were administered normal saline. Animals were provided free access to food and water for 4-6 hours after the administration of ulcerogenic substance and the same procedure was repeated for three days. On the day 4, animals of Group I (normal) and II (diseased group) were decapitated and stomach was dissected out. Stomach was incised along the greater curvature for gross examination and identification of ulcerative lesions. The tissues with exposed inner surface were photographed and preserved in 10% formalin for histopathological examination. Group III and IV were treated with 50 and 100 mg/kg doses of ulcerene, respectively 12 hourly, for next seven days. Whereas, Group V received the standard drug. On the day 11, animals were decapitated and stomach was incised for gross examination to observe effects of treatment on ulcer lesions. Tissues were photographed and presser-ved in 10% formalin for histopathological evaluation.

Calculation of the extent of gastric ulcer

The intensity of ulcer lesions was evaluated by gross examination of stomach photographs and ulcer score was assigned. After observing the intensity of lesions, ulcer index and %cure were calculated by the formula used previously (Desai et al., 1999).

Ulcer index =

(arithmetic mean of intensity in a group/total number of animals) + (number of ulcer positive animals/total number of animals) × 2

%Cure = (UI control − UI treated)/(UI control) × 100

0 = No ulcer, 1 = Mild redness, 2 = Superficial lesions, 3= Deep lesions, 4 = Penetrated ulcers/bleeding

In vitro experiments

All experiments were performed according to the protocol previously followed (Naz et al., 2016). Rabbit jejunum was removed out, after surgical opening of the abdominal cavity and was kept in Tyrode's solution. Approximately 2-3 cm length of jejunum was suspended in tissue organ bath, containing 20 mL Tyrode's solution, supplied with oxygen and warmed at 37°C. Each tissue was equilibrated for 20 min and then stabilized by repetitive (3-5 times) administration of acetylcholine (0.3 µM) with subsequent Tyrode's washing. These experimental conditions permit the investigation of drugs effect on spontaneous jejunum contractions. Isotonic responses of intestine were documented through Bioscience transducer, connected to a computer via a data acquisition system: Power Lab (AD Instruments, Australia).

Preliminary phytochemical analysis

The qualitative phytochemical screening of crude extract of *K. integra* was conducted to evaluate the presence of different chemical classes as previously followed (Naz et al., 2016).

Results

Preliminary phytochemical analysis

Preliminary phytochemical analysis was done for the detection of different phytochemical classes in the crude extract of ulcerene. It was found positive for alkaloids, carbohydrates, proteins, phenols, tannins and saponins.

Effect in ethanol-induced ulcer (in vivo experiment)

In diseased group, the ulcer score was 4. While in ulcerene (50 and 100 mg/kg) -treated group, the ulcer score was significantly ($p<0.001$) reduced to 0.8 and 0.4, respectively. Sucralfate (positive control group) significantly ($p<0.001$) reduced the ulcer score to 0.2. Ulcer index of the diseased group was 3.6. However, ulcer index of ulcerene 50, 100 mg/kg and sucralfate-treated groups was found to be 1.92, 0.96 and 0.48, respectively.

Table I

Ulcer score, ulcer index and percentage cure of different treatment groups in ulcerative rats

	Ethanol (1 mL/200 g)-induced ulcer			Aspirin (200 mg/kg)-induced ulcer			Stress-induced ulcer		
	Ulcer score	Ulcer index	%Cure	Ulcer score	Ulcer index	%Cure	Ulcer score	Ulcer index	%Cure
Diseased	4.0 ± 0.0	3.6		4.0 ± 0.0	3.6		4.0 ± 0.0	3.6	
Ulcerene (50 mg/kg)	0.8 ± 0.2[a]	1.9	46.7	1.0 ± 0.0[a]	2.4	33.3	1.2 ± 0.2[a]	2.4	31.1
Ulcerene (100 mg/kg)	0.4 ± 0.2[a]	1.0	73.3	0.4 ± 0.2[a]	1.0	73.3	0.8 ± 0.2[a]	1.9	46.7
Sucralfate (100 mg/kg)	0.2 ± 0.2[a]	0.5	86.6						
Misoprostol (100 mg/kg)				0.4 ± 0.2[a]	1.0	73.3			
Ranitidine (50 mg/kg)							0.4 ± 0.2[a]	1.0	73.3

[a]p<0.001, one way ANOVA, followed by Dunnett's test

The curative percentage was also calculated and it was found that ulcerene 50 and 100 mg/kg cured 46.7% and 73.3%, respectively and 86.6% was that of sucralfate (Table I).

Histopathological evaluation of gastric tissue showed chronic superficial gastritis and hyperplasia of the cells in the diseased group. In ulcerene 50 mg/kg, mild superficial gastritis was seen with subepithelial tissue infiltrated by the inflammatory cell infiltrate. In 100 mg/kg, mild superficial gastritis was seen, whereas in sucralfate treated group normal gastric subepithelial tissues and no inflammation or congestion in the tissues, comparable to control group was seen (Figure 1).

Effect in aspirin-induced ulcer

In diseased group, ulcer score was found to be 4.0. While in ulcerene (50 and 100 mg/kg) -treated group, the ulcer score was significantly (p<0.001) reduced to 1 and 0.4, respectively. Whereas misoprostol (positive control group) also significantly (p<0.001) reduced the ulcer score to 0.4. Ulcer index of the diseased group was 3.6. However, ulcer index of ulcerene 50, 100 mg/kg and misoprostol-treated groups was found to be 2.4, 0.96 and 0.96, respectively. The curative percentage was also calculated and it was found that ulcerene 50 and 100 mg/kg cured 33.4 and 73.3%, respectively and 73.3% was that of misoprostol (Table I).

Histopathology of the diseased tissue showed the gastric mucosa focally eroded and partially covered by benign columnar epithelium, subepithelial tissues infiltrated by chronic inflammatory cells with macrophages. It shows superficial gastritis with benign ulceration. In ulcerene 50 mg/kg, congestion and mild chronic inflammatory cell infiltrate in lamina propria was observed. In ulcerene 100 mg/kg, mild congestion with mild superficial gastritis was seen. Misoprostol-treated group also showed mild congestion in the mucosa and mild superficial gastritis comparable to that of 100 mg/kg (Figure 1).

Effect of stress-induced ulcer

In diseased group, ulcer score was found to be 4.0. While in ulcerene (50 and 100 mg/kg) treated group, the ulcer score was significantly (p<0.001) reduced to 1.2 and 0.8, respectively. Whereas ranitidine (positive

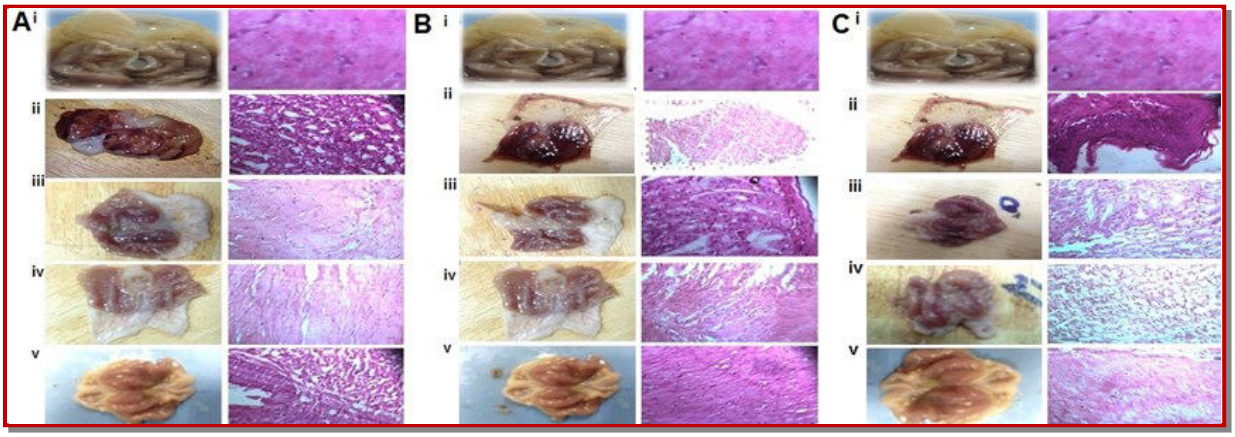

Figure 1: Showing gross view photographic and histopathological images of rat's stomach in (i) control group, (ii) diseased group, (iii) ulcerene (50 mg/kg) (iv) ulcerene (100 mg/kg) and (v) standard drug treated groups in (A) ethanol- (1 mL/200 g), (B) aspirin- (200 mg/kg) and (C) stress-induced ulcerative rats

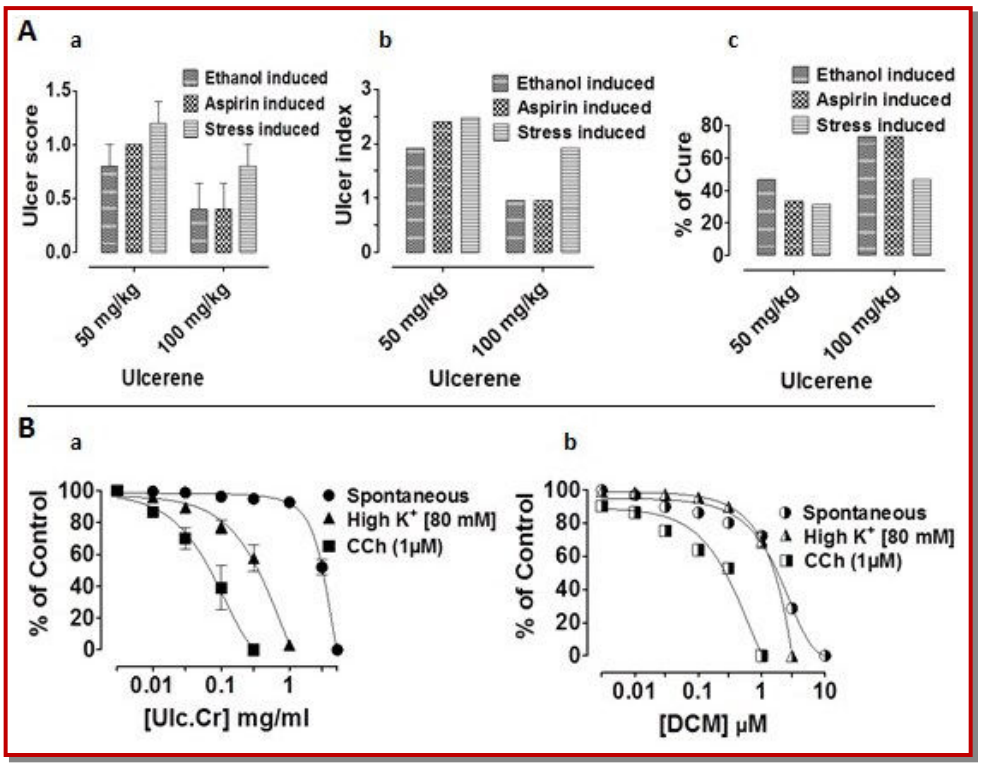

Figure 2: (A) Bar-chart showing comparison of (a) ulcer scores, (b) ulcer index and (c) percentagecure of ulcerene (50 and 100 mg/kg) treated groups in different ulcer models and (B) relaxant effect of (a) Ulc.Cr (crude extract of ulcerene) and (b) dicyclomine (DCM) on spontaneously contracting and of high K^+ [80 mM] or CCh (1 μM) contractionof rabbit jejunum preparations. Values expressed with mean ± SEM., n=3–5

control group) also significantly ($p<0.001$) reduced the ulcer score to 0.4. Ulcer index of the diseased group was 3.6. However, ulcer index of ulcerene 50, 100 mg/kg and ranitidine-treated groups was found to be 2.48, 1.92 and 0.96, respectively. The curative percentage was also calculated and it was found that ulcerene 50 and 100 mg/kg cured 31.1% and 46.7%, respectively and 73.3% was for ranitidine (Table I).

Histopathological findings showed gastric mucosa covered by benign columnar epithelium with congestion in lamina propria. Gastric mucosal congestion and hyperplasia of the chief cells were observed. In ulcerene 50 mg/kg, gastric mucosa covered by benign columnar epithelium with mild congestion was seen. The subepithelial tissue is infiltrated by mild chronic inflammatory cells. In ulcerene 100 mg/kg, very mild superficial gastritis was seen. Ranitidine-treated group showed mild superficial gastritis (Figure 1).

A dose-dependent decrease in the ulcer score and index of ulcerene was observed. When comparing the results of the different models it was observed that ulcerene was most effective against ethanol-induced ulcer with highest percent cure (Figure 1).

Effect on intestinal motility (In vitro experiment)

Concentration-dependent spasmolytic effect of ulcerene was seen, when given in cumulative fashion (0.01-10 mg/mL), on spontaneously contracting isolated rabbit jejunum, similar to dicyclomine with EC_{50} value of 3.28 (95% CI, 3.12-3.44, n=3); 1.89 (1.69-2.11, n=3), respectively. A complete relaxation with ulcerene was also observed in high K^+ (80 mM) and carbachol (1 μM) induced jejunum contraction at 0.3 and 1 mg/mL, with EC_{50} value of 0.367 (95% CI, 0.317-0.424, n=3); 0.0672 (0.0463-0.0974, n=3), respectively, similar todicyclomine at 1 and 3 μM with EC_{50} value of 1.58 (0.973-1.924, n=3); 0.267 (0.188-0.380, n=3) (Figure 2).

Discussion

The study was aimed to evaluate the ulcerene, a polyherbal formulation, for the ulcer curative potential by applying *in vivo* models and *in vitro* techniques. The gastric ulcer models used were ethanol, NSAIDs and stress induced. Curative potential and probable mode of action were evaluated by examining gross appearance and histopathology. Whereas isolated rabbit jejunum was used for evaluation of possible spasmolytic activity to check the efficacy in duodenal ulcer.

Ethanol is identified as a major risk factor for development of gastric ulcers (Sener et al., 2004). It decreases discharge of bicarbonate, suppresses the production of gastric mucosa (Al Batran et al., 2013). This action

destroys the protective mucosal layer and renders the cells of stomach lining exposed to proteolytic and hydrolytic actions of gastric acid (Sener et al., 2004). Since ulcerene (50 and 100 mg/kg) significantly ($p<0.001$) reverted the damage to gastric layer, proposes its ulcer curative property that can be possibly mediated through cytoprotective action on gastric mucosa, as shown by sucralfate (100 mg/kg). Ethanol also contributes to gastric mucosal injury through enhanced intracellular accumulation of calcium that promotes the death of gastric epithelium cells (Massignani et al., 2009). Relaxation of high K^+ induced pre-contracted isolated rabbit jejunum by a crude extract of ulcerene, suggests the presence of calcium channel blocking activity, strengthen the effectiveness of ulcerene in ethanol induced ulcers.

Prostaglandins play the protective role in the stomach by regulating mucosal secretion (Coblijn et al., 2014) and by decreasing gastric acid secretion (Yedgar et al., 2007). NSAIDs inhibit cyclooxygenase enzymes (COX-1 and COX-2) in turn decreasing bicarbonate and mucus secretion. It also reduces blood flow of mucosa, impairs platelet aggregation and alters microvascular structures, damaging epithelium and causing internal bleeding (Takeuchi, 2012; Wallace et al., 2000). There-fore, NSAIDs suppress prostaglandins that results in mucosal injury and subsequent gastric ulcer. Groups treated with ulcerene (50 and 100 mg/kg) showed a significant ($p<0.001$) decrease in mean ulcer score, similar to misoprostol (100 mg/kg). So, it can be suggested that ulcerene promotes endogenous prostaglandin synthesis and maintains mucosal protection resulting in ulcer curative activity.

Histamine released during stress results an increase in gastric acid secretion and a decrease in mucous production, is known to be responsible for stress-induced ulcer (Jia et al., 2007). However, a significant reversal of ulcer score and ulcer index by ulcerene (50 and 100 mg/kg), suggests its ulcer curative potential, that can be related to the blockade of H_2 receptors similar to ranitidine (50 mg/kg). During stress, increased vagal tone also stimulates gastrointestinal motility (Caso et al., 2008) and decreases the quality of gastric mucosal lining also (Panda and Sonkamble, 2012). Since ulcerene relaxed carbachol (1 µM) induced pre-contracted isolated rabbit jejunum, supports the presence of anti-cholinergic activity that strengthens ulcer curative potential of ulcerene.

Effectiveness in the duodenal ulcer of antiulcerogenic agent is very much dependent on its gastric motility (Mahattanadul et al., 2011). It would perform better healing effect if the intestinal transit time of the drug is increased. Inhibition of spontaneous contractions of isolated rabbit jejunum indicates the presence of spasmolytic activity (Mushtaq et al., 2015; ur Rahaman et al., 2013). For the evaluation of mechanism involved in spasmolytic activity, high K^+ and carbachol-mediated pre-contracted isolated rabbit jejunum were treated with ulcerene. A pronounced relaxation was observed, more effective against carbachol than high K^+, similar to dicyclomine: A dual blocker of muscarinic receptor and Ca^{2+} channel (Downie et al., 1977). Therefore, a non-specific spasmolytic mechanism is most probably involved, through Ca^{2+} channel blocking activity and the additive effect of anticholinergic constituents present in the crude extract of ulcerene.

Conclusion

Ulcerene can be recommended as an alternative remedy for the treatment of gastric ulcer that cures gastric ulcers through different mechanisms.

Ethical Issue

Experiments were performed according to the instruction of Institutional Animal Ethical Committee, constructed under the guidelines of National Research Council.

Conflict of Interest

No conflict of interest exists.

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